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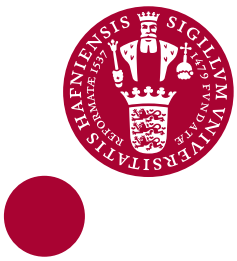

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Pires dos Santos, Teresa M S; Pors, Susanne Elisabeth; Nielsen, Ole Lerberg; Christensen, Henrik; Petersen, Andreas; Larsen, Jesper; Bisgaard, Magne

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ASSOCIATION MAROCAINE DE PATHOLOGIE AVIAIRE

50th Anniversary

Detection of *Enterococcus faecalis* in chicken tissue sections by fluorescent 16S rRNA in situ hybridization

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Enterococcus spp. are important emergent pathogens. Most of reported cases of infection have involved bacterial endocarditis, hepatic granulomas, and occasionally acute septicemia. *Enterococcus faecalis* has been the most common isolate and the one most consistent in producing bacterial endocarditis in experimental infections via the intravenous route. The aim of this investigation was to develop a fluorescence in situ hybridization (FISH) assay to facilitate specific identification of E. faecalis in formalin fixed, paraffin embedded chicken tissue sections. An oligonucleotide probe specific for E. faecalis 16S rRNA was selected from the literature (ENF191). The specificity of the probe was tested with pure cultures of related organisms which could pose a differential diagnostic problem. Uninfected tissue was tested as a negative control. Fourteen hens of the type White Plymouth Rock were subsequently used for the experiment at the age of 60 weeks. Birds were inoculated with 109 CFU ml-1 of E. faecalis strain 54869-1997 and sacrificed 14 days later. Samples were processed and tissue sections were hybridized and examined by fluorescence microscopy. The co-occurrence of bacteria and histological lesions were evaluated by reading haematoxylin-eosin stained sections. A strong fluorescent signal was detected from all E. faecalis strains tested, whereas no detectable signals were obtained from other enterococcal species with ENF191 probe. Likewise no signal was obtained from non-enterococcal species. Hybridization of the eubacterial probe, EUB338, with all the strains included showed a signal intensity corresponding to the level observed with ENF191 hybridized with strains of E. faecalis. None of the sense probes bound to the tissue used as negative control. The infection experiments revealed a similar signal to the one detected in the control studies. The signal distribution corresponded to the microcolonies and basophilic aggregates of bacteria seen in the valve in the HE-stained sections. Tissue hybridization with the eubacterial probe, EUB338, always revealed a pattern similar to that obtained with ENF191, whereas hybridization with the unspecific, anti-sense probe, NON338 was negative for infected and non-infected control tissues. In conclusion, the assay developed was able to detect single cells of E. faecalis in situ in the respective tissue samples. This conclusion is supported by the observation that no signal was detected from control studies that contained organisms other than E. faecalis. The present protocol is readily adaptable to detect E. faecalis in natural infections and should prove to be a useful tool in identifying infective colonization processes.